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Action of Drugs Containing Basic Nitrogen on d-Amino Acid Dehydrogenase.

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A complete analysis of the actions of a drug has come to include information regarding the effect of the drug upon the metabolism of surviving tissue slices and upon the catalytic properties of isolated enzyme systems. In this paper the effects of certain drugs upon the conversion of d-alanine to pyruvic acid with concomitant utilization of oxygen in the presence of the enzyme d-amino acid dehydrogenase are reported. The drugs studied contained basic nitrogen and were chosen to include different types of functional nitrogen. Krebs[1] has already reported the inability of veronal to modify the catalysis of deamination of d-amino acids by d-amino acid dehydrogenase in experiments in which the barbiturate was used in a buffer system. Klein and Kamin[2] reported the failure of a large group of compounds containing nitrogen to modify the action of d-amino acid dehydrogenase.

Method. The enzyme was prepared by the method of Krebs.[1] The minced hog kidneys came in contact with acetone within 15 minutes after the death of the animals. The extracts were prepared by shaking 1 g of the dried powdered kidneys in 40 ml of distilled water for 10 minutes. After centrifugation for 10 minutes the supernatant extract was pipetted off. It contained 4.04 mg protein per ml and was as active as similar extracts reported by other workers.

The utilization of oxygen in the deamination of d-alanine in the presence of the extract was measured by the method of Warburg,[3] in the presence of oxygen at 37°C (±.02°). The extract was buffered with Na_2HPO_4 in the reaction vessels. After attainment of equilibrium, substrate, also buffered with Na_2HPO_4, was added to the control vessel from the side arm. The final concentration of dl-alanine was 4.4×10^{-2} M; of phosphate, 0.034 M. In the case of the experimental vessels similarly buffered solutions containing both dissolved substrate and drug were introduced from the side arms.

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[1] Krebs, H. A., *Biochem. J.*, 1935, **29**, 1620.

[2] Klein, J. R., and Kamin, H., *J. Biol. Chem.*, 1941, **138**, 507.

[3] Warburg, O., *Über den Stoffwechsel der Tumoren*, Berlin, 1926.

Final experimental concentrations of substrate were 4.4×10^{-2} M; of phosphate, 0.034 M; and of drugs, 2×10^{-3} M. Such concentrations of drug have been found acceptable in our[4] studies of the action of drugs on surviving tissue slices and are in the same range as those of certain therapeutic agents in the blood-stream during medication. Furthermore such concentrations do not, at the pH finally attained, introduce too great danger of precipitation either of drug or of dispersed protein. The values of final pH for each system will be found in the tabulated results.

Our experience with presumably pure substrate disclosed that the quantitative and even the qualitative nature of the action of quinidine on the dehydrogenase depended upon the source of the sample of d-alanine. For example, with one substrate sample, quinidine augmented the catalytic action of d-amino acid dehydrogenase while, with another, it depressed it. These observations could be reproduced at will under rigorously controlled conditions in which checks did not vary by more than 2%. To arrive at results which represented the true action of the drug it was necessary to use samples of d-alanine purified by 4 recrystallizations from aqueous alcohol. Continued purification of each of 3 samples of dl-alanine led to a decrease in and finally to a disappearance of discrepancy in behavior. That is, after final recrystallization each sample of alanine yielded a reaction which could be qualitatively and quantitatively reproduced with the other samples. The commercial samples were procured from Eastman Kodak Co. and from Hoffman-LaRoche Co. The other sample was prepared in this laboratory by the acetaldehyde-ammonium cyanide reaction. The last preparation recrystallized 4 times after its original presumable purification served as the substrate on which we report. It is important to note that results obtained from an improperly purified substrate might be ascribed by the observer to properties of the enzyme rather than to those of the substrate except in those cases where the action of a drug chanced to disclose the real nature of the situation. We are not yet prepared to state why substrates from different sources produce such variations in results.

Since it is not possible to secure final conditions in which concentrations of all electrolytes, concentrations of substrate, and pH are identical, it was elected to gain the former identities and to make interpretation in the light of slight differences in final pH. For example, with the system containing quinidine sulfate the average

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[4] Rice, J. C., and Brazda, F. G., *Proc. Am. Physiol. Soc.*, p. 234, Chicago, Ill., April, 1941.

of the initial and final pH values was 7.98 (glass electrode), while that of the control was 8.04. In spite of the fact that the latter pH is more nearly optimal the systems containing quinidine led to a greater utilization of oxygen by 9%. This augmentation is easily reproducible; it represents the limiting value of the activity of systems in which substrates from different sources and in different states of purity are used; it transcends the variation which arises from the method as we use it by 7%. It must unquestionably represent a property of quinidine sulfate as related to the action of d-amino acid dehydrogenase. Its significance rests upon the results from at least 20 determinations.

Results. Calculated in terms of gross oxygen utilization over a one-hour period in control situations our extracts demanded 500-600 cmm of O_2 per 2 ml aliquot on different occasions. This range of oxygen utilization arises from difference in fineness of powdering, unavoidable variable hysteresis of each dispersed extract, and the effect of time in causing deterioration of the powdered kidney. The variability is not related to the interpretation of experimental results because the action of a drug was compared with controls which utilized the identical extract. The results are expressed as percentage augmentation or depression and are found in Table I. Determinations for each drug were reproduced at least 4 times except those for quinidine which demanded the more extensive treatment already described above.

The tabulated results indicate an augmentation by veratrine amounting to 6%. This exceeds by 4% the variation which we are accustomed to expect with this method. Bernheim and Bernheim[5] have reported that veratrine inhibits the oxidation of proline by

TABLE I.

Drug	Effective concentration	Action on catalysis by d-amino acid dehydrogenase	Avg of Initial and final pH
Control	—	—	8.04
Quinidine (as sulfate)	2×10^{-3} M	9% augmentation	7.98
Veratrine (as sulfate)	” ”	6% ”	7.94
Ephedrine (as sulfate)	” ”	3% ”	7.84
Sulfanilamide	” ”	3% ”	7.84
Pilocarpine (as hydrochloride)	” ”	3% inhibition	7.65
Morphine (as sulfate)	” ”	No change	7.81
Physostigmine (as sulfate)	” ”	” ”	7.75
Quinine (as sulfate)	” ”	” ”	7.98
Riboflavin	Kidney extract saturated with riboflavin	” ”	8.01

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[5] Bernheim, F., and Bernheim, M. L. C., *J. Pharm. and Exp. Therap.*, 1933, **48**, 73.

liver tissue. It is possible that with higher concentrations of veratrine this might have happened to the action of d-amino acid dehydrogenase on d-alanine. With such concentrations there might have arisen, however, the hazard of incipient precipitation of either protein or drug, a hazard which we strove to avoid.

Quinidine augmented the reaction more strongly than veratrine. Quinine, however, failed to cause any measurable modification of the utilization of oxygen. It must be pointed out, however, that since incipient precipitation of quinine resulted in every experiment the critical concentration may not have been reached. Since the utilization of oxygen in the system containing quinine was not different from that in the control vessels, the material precipitated could hardly have been enzyme-bearing proteins. It was observed repeatedly in experiments employing pH values around 8.0 and a milieu of PO_4 ion that it is not possible to exceed the concentrations of the sulfate of quinine and quinidine which we employed. The stereoisomeric difference between quinine and quinidine is sufficiently fundamental to produce the difference observed.

Conclusions. If one excludes the effects on pH which are produced in the reacting systems by the presence of drugs, the results indicate that ephedrine, morphine, physostigmine, pilocarpine, riboflavin, and sulfanilamide do not modify the catalysis of the dehydrogenation of d-alanine by d-amino acid dehydrogenase. The augmentations produced by quinidine and veratrine, although small, are significant.

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A New Growth Factor for Hemolytic Streptococci.

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Reports have appeared on cultivation of *Str. hemolyticus* in synthetic or semisynthetic media to which growth accessory factors were added. Woolley and Hutchings[1] grew strains of Lancefield's B and D types on a medium consisting of amino-acids, glucose, salts and riboflavin, pantothenic acid, pyridoxin and reduced iron; groups

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[1] Woolley, D. W., and Hutchings, Brian, L., *J. Bact.*, 1940, **39**, 287.